Chapter

# Antimicrobial Usage for the Management of Mastitis in the USA: Impacts on Antimicrobial Resistance and Potential Alternative Approaches

*Benti D. Gelalcha, Getahun E. Agga and Oudessa Kerro Dego*

## Abstract

Mastitis is the most frequently diagnosed disease of dairy cattle responsible for the reduction in milk quantity and quality and major economic losses. Dairy farmers use antibiotics for the prevention and treatment of mastitis. Frequent antimicrobial usage (AMU) undeniably increased antimicrobial resistance (AMR) in bacteria from dairy farms. Antimicrobial-resistant bacteria (ARB) from dairy farms can spread to humans directly through contact with carrier animals or indirectly through the consumption of raw milk or undercooked meat from culled dairy cows. Indirect spread from dairy farms to humans can also be through dairy manure fertilized vegetables or run-off waters from dairy farms to the environment. The most frequently used antibiotics in dairy farms are medically important and high-priority classes of antibiotics. As a result, dairy farms are considered one of the potential reservoirs of ARB and antimicrobial resistance genes (ARGs). To mitigate the rise of ARB in dairy farms, reducing AMU by adopting one or more of alternative disease control methods such as good herd health management, selective dry-cow therapy, probiotics, and others is critically important. This chapter is a concise review of the effects of antimicrobials usage to control mastitis in dairy cattle farms and its potential impact on human health.

**Keywords:** antimicrobial resistance, bovine mastitis, intramammary infection, antimicrobials, mastitis, bovine, dairy cattle

## 1. Introduction

Since the discovery of antibiotics, microbes have continued to uncover new ways to survive and thrive in the presence of antibiotics [1]. In recent years, the emergence and spread of antimicrobial resistance (AMR) worldwide have increased at an alarming rate [2]. AMR has been detected almost as quickly as newer antibiotics were developed and used [3]. Mastitis, an inflammation of the mammary gland, mainly caused by bacteria, is the most frequent reason for antibiotic use in dairy cattle. Mastitis causes significant economic losses to the dairy industry directly through a reduction in milk yield and quality and indirectly by

increasing the cost of its management [4]. The indirect cost includes heavy use of antibiotics, which contributes to the occurrence of AMR. In addition, some AMR mastitis pathogens can pose public health threats through the consumption of milk and milk products [5].

The rise in AMR occurs mainly due to the imprudent use of antimicrobials which increasingly undermines the sustainable use of antimicrobials. Studies reported that the amount of antimicrobials used (AMU) to treat clinical and subclinical mastitis accounts for nearly twice the quantity of antibiotics used for all other health problems in dairy cows [6, 7]. The United States Department of Agriculture (USDA), National Animal Health Monitoring System (NAHMS) survey of 2013 reported a 24.8% clinical mastitis in all cows involved [8]. The majority (87.3%) of the cows with clinical mastitis were given antibiotic treatment. Nearly three-fourths of the farms (73%) used cephalosporins, 34.4% used first-generation cephalosporins (FGCs), and 38.6% of them used third-generation cephalosporins (TGCs). The NAHMS also reported that out of 21.4% of cows treated for mastitis, the primary treatments given were TGCs (50.7%), lincosamide (24.7%), and FGCs (15.2%). The same report showed that there are seven approved intramammary (IMM) antimicrobial products in the United States but no systemic products for treating clinical mastitis except limited extra-label usage of some products. While one approved IMM antimicrobial product is classified as a lincosamide (pirlimycin) and six IMM antimicrobial products are classified as beta-lactams. The beta-lactams that are used as IMM products include FGCs (cephapirin) and TGCs (ceftiofur), aminopenicillins (amoxicillin and hetacillin), penicillin G, and penicillinase-resistant penicillins (cloxacillin) [9].

Another most common AMU is for dry cow therapy (DCT). Dairy cows are susceptible to intramammary infection (IMI) during the early and late dry period [10–12]. To prevent IMI during the dry period, the National Mastitis Council (NMC) recommends IMM of long-acting IMM antibiotics, also known as dry cow therapy (DCT), as a prophylactic control measure for the management of mastitis. The DCT is routinely used at the end of lactation to cure existing subclinical mastitis so that it will not be carried over to the next lactation and to prevent new infections during the dry period [13]. According to the 2014 NAHMS of dairy herds study, 93% of cows in the U.S. received DCT. Among the operations that used DCT, more than half (58.1%) of them used cephapirin benzathine followed by ceftiofur 27.9%, and procaine penicillin G and dihydrostreptomycin sulfate combination (24.5%). A recent study also reported that beta-lactam antibiotics such as cephapirin, ceftiofur, and penicillin are the top three antibiotics used for DCT on U.S. farms [14].

Although total AMU in the U.S. cattle production, including dairy farming, is lower than that of other food animals such as pigs, most of the antibiotics used are important to treat infections in humans. Of all antibiotics classes approved for use in U.S. dairy cattle, at least eight are medically important (**Table 1**). These antibiotics used in both dairy and human medicine include aminoglycosides, cephalosporins, fluoroquinolones, lincosamides, macrolides, penicillins, sulfonamides, and tetracyclines [19]. These antibiotics are also used to treat other diseases of dairy cattle, such as respiratory and reproductive diseases and foot infections [7]. Some of these antibiotics are categorized by the World Health Organization (WHO) as critically important ones. Quinolones (enrofloxacin and danofloxacin) and extended-spectrum beta-lactams such as third-generation cephalosporins, which are heavily used in U.S. dairy farms for the treatment of mastitis, are considered as "highest priority critically important" classes of antibiotics [19]. The use of these antibiotics in dairy farms can exert selection pressure that may lead

| Antimicrobial class | Antimicrobial agent | Indications | Importance for human medicine | References |
|---|---|---|---|---|
| Cephalosporins | Ceftiofur<br>Cephalothin<br>Cephapirin | DCT, BRD, mastitis, and metritis | Critically important | [8, 15–18] |
| Fluoroquinolones | Danofloxacin, Enrofloxacin | BRD | Critically important | [16, 17] |
| Aminoglycosides | Amikacin, Clindamycin<br>Gentamicin, Apramycin<br>Kanamycin, Neomycin | DCT, feet infections | Critically important | [16–18] |
| Penicillin | Amoxicillin/Clavulanic acid<br>Ampicillin<br>Penicillin<br>Cloxacillin | DCT, mastitis, metritis, and other local infections | Important | [14, 16, 17] |
| Sulfonamide | Sulfamethoxazole, Sulfadimethoxine, Sulfisoxazole, Trimethoprim/ Sulfamethoxazole, Sulfamethoxazole/ Sulfisoxazole | Calf diarrhea | Highly important | [16–18] |
| Macrolides | Erythromycin, Tilmicosin<br>Tulathromycin, Tylosin<br>Tulathromycin and Gamithromycin, Tilmicosin, | BRD, foot rot, and metritis | Critically important | [16–18] |
| Amphenicols | Florfenicol | BRD | Highly important | [16–18] |
| Tetracyclines | Chlortetracycline<br>Oxytetracycline<br>Tetracyclines | BRD, metritis, bacterial scours, and eye infection | Highly important | [16–18] |
| Lincosamide | Pirlimycin, Lincomycin | DCT, Mastitis, BRD, and feet infections | Highly important | [16, 17] |

*BRD: Bovine respiratory disease; and DCT: Dry cow therapy.*

**Table 1.**
*Major antimicrobial classes used in the U.S. dairy cattle and their medical importance according to WHO classification.*

to the emergence and spread of AMR pathogenic, opportunistic, and commensal bacteria from dairy farms to humans. Transmission may occur through direct contact between cattle and humans or indirectly through the food chain (milk and meat). The horizontal transfer of resistance genes may occur from bacteria of dairy cattle origin to human commensal or pathogenic bacteria in the gut [20]. Thus, the development of AMR that arises from the AMU in dairy farms could seriously impact the management of infectious diseases in the human population using antibiotics [21].

## 2. Antibiotics use in dairy farms and their implication to human health

There is considerable evidence that supports the view that the development of AMR in food animals such as dairy cattle is linked to the emergence of AMR bacteria that infected humans [22–24]. As one of the major consumers of antibiotics, dairy cattle production farms are likely to contribute to the rise of AMR bacteria in humans. Studies from outside of the U.S. [25–27] showed direct transmission of AMR from dairy cattle to humans through contact on farms or through indirect routes. The most common route of the spread of AMR bacteria and their resistome from dairy cattle farms to humans could be indirect through the food chain. In the U.S., the CC97 methicillin-resistant *S. aureus* (MRSA), the human pandemic clone, which claims the lives of thousands of people every year, was suggested to be originated from the dairy farm [28].

According to the U.S. centers for disease control and prevention (CDC), about 22% of infections (440,000 cases) caused by antibiotic-resistant pathogens in the U.S. are from a food of animal origin, such as milk [29, 30]. Most of these bacteria could be normal microflora that colonizes the gastrointestinal tract of the animal [24], but they could be pathogenic for humans or may also be commensal but may transfer resistance genes to other foodborne pathogens in the human gastrointestinal tract [23]. Additional routes of transmission of AMR bacteria and their resistome to humans is through contaminated dairy farm environments and other wastes entering the environment [31].

Multiple studies have linked the outbreak of foodborne AMR pathogens to animal and their products, including milk [25–27, 32]. Despite these reports, it should be noted that direct proof for AMR transmission through foods of animal origin or directly through contact is limited, especially from dairy cattle [33]. In the U.S., strong evidence for transmission of AMR isolates between dairy cattle and humans is not yet proven. Previous reviews that attempted to discern any linkage between AMU in dairy cows and AMR development in veterinary and human pathogens showed the absence of scientific proof to support this assumption [34]. However, there is ample evidence that the use of antibiotics in food-producing animals contributes to increased AMR [35]. Published literature showed that the risk of getting an infection from AMR zoonotic dairy pathogens seems less likely [36].

However, the absence of direct evidence of AMR bacteria or resistant determinant transmission does not mean there is no transmission between dairy cattle and humans. For instance, the current and future risk of acquiring AMR bacteria from milk is an important human health concern as the consumption of raw milk is increasing in some states in the U.S. [32]. Due to the presence of antibiotic-resistant foodborne or zoonotic bacteria in raw milk [29, 30], *an increasing trend in the consumption of raw* milk in the U.S. and other countries indicates public health risk [37]. Similarly, AMR bacteria present on meat from culled dairy cows should also be seen as an important human health risk since it can cause life-threatening infection if undercooked meat is consumed [34]. It is also unknown if pasteurization of milk or proper cooking of meat will prevent the AMR gene transfer especially in the gastrointestinal tract where horizontal gene transfer may occur.

### 2.1 Antimicrobial resistance in mastitis pathogens

Antibiotics are regularly used for the prevention and treatment of mastitis in dairy cows. Some review articles showed such uses had not been associated with a high risk of developing resistance in mastitis-causing pathogenic bacteria [7, 34]. The previous review on the impact of antibiotic use in adult dairy cows on antimicrobial resistance of veterinary and human pathogens concluded that common

AMU in dairy farms did not lead to the widespread occurrence of resistance among mastitis pathogens against antibiotics frequently used in dairy production [34]. Nevertheless, there is no doubt that AMU in food-producing animals such as dairy cows contributes to the rise in AMR [7]. Recently Abdi et al. [38] reported a high prevalence (34.3%) of resistant *S. aureus* isolates from different dairy farms in Tennessee, U.S. suggesting a potential increasing trend of antimicrobial resistance in *S. aureus* isolates against some antibiotics.

Only a handful of studies investigated the impact of treatment of clinical or subclinical mastitis on AMR development. A controlled study by Levy et al. [39] measured AMR changes after antimicrobials were administered to a host; however, this study lacks mastitis treatment procedures [7]. However, some studies showed that AMU for mastitis treatment is linked to AMR development and changes in the diversity of mastitis pathogens [40, 41]. Pol and Ruegg [4] found a positive relationship between AMU such as pirlimycin, ampicillin, erythromycin, and tetracycline and increased resistance among gram-positive mastitis pathogens. Another U.S. study also reported a higher proportion of resistant mastitis pathogens recovered from conventional dairy farms than organic dairy farms [42], suggesting the effect of AMU.

## 3. Alternative approaches for the management of mastitis

There were no specific AMU data collected from U.S. dairy farms. Thus, it is not possible to know the doses of each antibiotic given to dairy cattle, the length of the treatment, and the diseases for which antibiotics were prescribed. However; there is no doubt that antibiotics have been administered for a considerable proportion of dairy cattle's lifetime in a farm, and dairy farm consumes a huge quantity of antibiotics, especially those of the medically important ones. The United States Food and Drug Administration (FDA) report showed more than 16,155 kg of medically important antimicrobials intended for IMM therapy were sold in 2019 [19].

The major concern is the use of critically important antibiotics for human medicine in dairy farms such as third-generation cephalosporins and fluoroquinolones. Both qualitative and quantitative studies that analyzed the risk of AMR in food animals such as dairy farms indicated that the continued use of these antimicrobials would increase the number and types of AMR bacteria and worsen the public health and animal health issues in the U.S. and beyond [43]. It is no longer deemed appropriate that antibiotics should be the only remedy to prevent disease, especially when other alternative disease control measures exist. Thus, it is important to look for potential alternative strategies that help to reduce AMU and prevent disease without heavily relying on antibiotics [7]. Some of the alternative approaches that can be explored to mitigate the rise of AMR bacteria include but are not limited to selective dry-cow therapy (SDCT) [44], good herd health management [45], vaccination [46], phage therapy [47], probiotics [48] antibacterial peptides [49], and nucleic acid-based antibacterial treatments such as CRSPR-Cas system [50].

### 3.1 Selective dry cow therapy (SDCT)

The number one reason for AMU in the U.S. dairy industry is to control mastitis. Studies showed that almost all U.S. dairy farms treat all cows in the farm (blanket dry cow therapy-(BDCT) with long-acting antibiotics at drying off to prevent mastitis during the dry period. The ideal dry period, the period between the end of the current lactation and the beginning of the next, for a profitable dairy producer is usually 60 days or 8 weeks [51]. A USDA survey of dairy farms reported that 85%

of conventional dairy farms used BDCT [15]. A study suggests that BDCT accounts for approximately one-third of the total AMU on conventional dairy farms in the U.S. [52].

Selective dry cow therapy (SDCT), unlike BDCT, uses a specific strategy to avoid treating every cow with antibiotics at dry off. In SDCT, only animals with IMI or high somatic cell count or cows with a health record showing a high probability of developing mastitis receive antibiotics. A teat sealant is applied to all cows at drying off. Using an internal teat sealant prevents entry of mastitis pathogens and decreases the prevalence of clinical mastitis, reducing the need for treatments for clinical cases [44]. To determine cows that require SDCT, bacterial culture, or somatic cell count (SCC) data of individual animals are required. A cow with a composite milk high SCC of ≥200,000 cells/mL of milk indicated the presence of subclinical mastitis and is eligible for IMM antibiotic infusion [52]. Studies [44, 53] showed that internal teat sealants, alone or when used with antibiotics can decrease the risk of acquiring new IMI after calving by as much as 25%. Internal teat sealants lowered the risk of IMI by 73% compared with cows that do not have teat sealants suggesting its potential use for managing mastitis [44].

### 3.2 Evidence-based treatment of mastitis

Before administering antibiotics, it is crucially important to isolate and identify mastitis-causing agents from infected udder quarters. Bacterial isolation and identification should be attempted at least in large dairy operations to make an evidence-based decision on whether to use antibiotics. Some investigations have confirmed that on-farm bacterial identification can decrease AMU by as much as 50% [40] since the use of antibiotics is not justified in some infections caused by gram-negative bacteria such as *E. coli* with high "spontaneous self-cure" [54, 55]. Another study also showed that the majority of (as high as 57%) milk samples collected from quarters of cows with negative culture results did not have bacterial DNA [56] suggesting that environmental factors such as trauma or viral infection may trigger an inflammatory response or infected animal was already fully recovered during sample collection. Failure to detect bacterial DNA could be due to bacteria elimination from the udder quarters by the host immunity [7]. In general, the possibility of a natural cure without the use of antibiotics against some bacterial pathogens is well documented in dairy cattle [57–61], and it is an important alternative to consider before deciding on antibiotic use.

### 3.3 Good dairy herd health management

Dairy herd health management is an essential component in the fight against AMR. The objectives of herd health management are to prevent and control mastitis and other diseases using appropriate hygienic and management practices [62]. AMU can be reduced by improving hygiene, frequent physical examination of animals, regular herd testing for common diseases, and quarantining all-new replacement animals before mixing with the herd [45]. In addition, dairy cattle should be managed to reduce stress and promote their welfare and immunity by providing suitable housing (good ventilation, appropriate humidity, low stocking densities, and good hygienic practices). Studies showed that hard flooring, poor bedding, and overcrowded conditions increase the chance of cows developing mastitis, lameness, and respiratory diseases [63, 64]. All efforts made to maximize herd health and welfare will enhance the host immune function and considerably reduce mastitis and other common dairy cattle diseases, reducing the need for antibiotics [65].

### 3.4 Vaccination

Vaccination against mastitis pathogens is recommended as one of the most important strategies to prevent new infections, which in turn reduce AMU in dairy farms [46]. Vaccination against mastitis-causing bacteria induces the cow's immune response that fights against subsequent infection and disease. Effective vaccine enhances adaptive humoral (antibody-mediated Th2 immunity) and cellular (cell-mediated- Th1 and Th17 immunity) immunity against mastitis pathogen that inhibits or restricts bacterial growth or kills bacteria upon its invasion of a mammary gland. The enhanced immunity cures the infection or reduces the number of invading bacteria, which reduces pathogen damage to milk-producing tissues and lessens the clinical severity of disease and production losses [66].

Vaccines can be classified into inactivated/killed, live/attenuated, chimeric live attenuated, subunit, and nucleic acid-based (DNA or mRNA) vaccines, each with advantages and disadvantages [66]. Live vaccines contain attenuated disease-causing agents capable of replicating within the host but do not cause disease because of attenuated pathogenicity. Modified live vaccines (MLV) are usually developed from the naturally occurring pathogen by (1) attenuation in cell culture, (2) use of variants from other species, and (3) development of temperature-sensitive mutants. Recombinant live attenuated vaccines include: (1) live attenuated vectored vaccines- pathogen's antigenic parts incorporated into a harmless carrier virus or bacteria, (2) chimeric live attenuated vaccines—genes from the target pathogen substituted for similar genes in a safe, but closely related organism, and (3) nucleic acid (DNA or mRNA) vaccines—a DNA vaccine is an immunogenic product encoding gene (DNA) cloned into a plasmid that can be injected into the host, where it will be transcribed and translated into an immunogenic product. The mRNA vaccine contains a messenger RNA (mRNA) molecule that encodes antigen that induces an immune response [67].

Inactivated/killed pathogen vaccines contain whole pathogens that have been inactivated with agents, such as phenol (bacteria) and formalin or beta-proprionolcatone (viruses). Inactivated/killed vaccines lack pathogenicity and can neither replicate nor spread between hosts and require multiple doses and regular boosters. The efficacy of inactivated/killed vaccines depends on the use of potent adjuvants. Bacterin is one of the killed/inactivated vaccines in which a suspension of killed whole bacterial cultures is used as a vaccine. Protein vaccines—include naturally produced proteins of pathogens and induce less injection site reactions than products containing the entire pathogen. Recombinant subunit vaccines—contain synthetically produced antigens that induce immunity to a specific pathogen. Adjuvants are one of the components of killed/inactivated vaccines that function to modulate and amplify the host immune response to the accompanying antigen and are critical to the success of inactivated vaccines.

Live-attenuated bacteria can multiply in the host, expressing a complete range of antigens [68]. However, the most important shortcomings of the live vaccine are their persistence in the animal body for an extended time, limited shelf life, potential for contamination, may cause abortion in pregnant animals, and safety concerns as the attenuated organism may revert to full virulence [69]. On the other hand, killed vaccines are safe, induce good colostral (lactogenic) immunity, have longer shelf lives but may interfere with passive immunity and are less immunogenic, and need adjuvants to enhance immune responses [70].

There is no effective vaccine against mastitis pathogens, and results of vaccine efficacy studies showed limited efficacy against mastitis-causing bacterial pathogens [66]. The most targeted udder pathogens for vaccine development include *S. aureus* [71–81], *Streptococcus uberis* [82, 83], *Streptococcus agalactiae* [66], and

*E. coli* [84–86]. Among mastitis pathogens, most vaccine trials were conducted against *S. aureus,* a major mastitis pathogen with a low cure rate by antibiotics, and remain undetected in the subclinical form in dairy cows [47, 87, 88]. Currently, there are two commercially available bacterin vaccines against *S. aureus* mastitis. These are lysigen® (Boehringer Ingelheim Vetmedica, Inc., St. Joseph, MO) in the United States and Startvac® (Hipra S.A, Girona, Spain) in Europe and some other countries. Several staphylococcal vaccine efficacy trials showed that vaccination with bacterin vaccines induced increased antibody titers associated with partial protection in the blood and milk in some studies [71, 74, 78, 81] or no protection at all in some other studies [72, 79, 80]. Neither of the two commercial vaccines against *Staphylococcus aureus* mastitis on the market, Lysigin®, and Startvac® [79] confers protection under field trials and controlled experimental studies [71–74]. Some studies reported that Lysigin® reduced somatic cell count (SCC), clinical mastitis, and chronic intramammary infection (IMI) [89–91], whereas other field-based studies concluded no such effect [72, 73, 75–77]. Similarly, some studies reported vaccination with Startvac®, reduced incidence, severity, and mastitis duration in vaccinated cows compared to non-vaccinated control cows [71, 74, 78]. Contrary to these observations, other studies failed to find an effect on improving udder health or showed no difference between vaccinated and non-vaccinated control cows [79, 80]. Overall, effective intramammary immune mechanisms against staphylococcal mastitis are still poorly understood.

Mastitis vaccine research has been conducted over the past several years, but to date, developing an effective vaccine has been a challenge due to the nature of the disease and the pathogens involved [92, 93]. For instance, an increased immune response may not always be beneficial in bovine mastitis unless increased immunity is followed by a decreased number of infecting pathogens, as the presence of a large number of bacteria in the presence of fighting immune cells is considered as an indication of mastitis which decreases milk quality [93]. Successful vaccination is challenging because the volume of milk present in the gland dilutes the number of immune effector cells available to fight off infection [92, 93]. In addition, fat and casein in the milk reduce the bactericidal abilities of the immune cells [93].

The development of an effective vaccine against mastitis pathogens is one of the sustainable alternatives to antibiotics. However, it may not be practically possible to develop an effective vaccine against all bacteria that cause mastitis [68]. Thus, combining effective vaccines with other infection control measures may considerably reduce the incidence of IMI and thereby reduce the need to use antibiotics [66].

### 3.5 Immunostimulants

Immunostimulants are compounds that activate any components of the host's innate immune system and help to enhance disease resistance. Immunostimulants directly stimulate innate immune responses by activating immune cells (phagocytes), complement system, and increased lysozyme activity [94, 95]. Currently, immunostimulants are increasingly used as an alternative to antibiotics [96]. Immunostimulants are broad ranges of substances including minerals (selenium and zinc); amino acids (leucine, arginine, and ubenimex); vitamins (A, E, C); plants and plant polysaccharides, bacterial components (β-glucan, peptidoglycan, lipopolysaccharide); hormones and hormone-like substances; nucleic acid preparations; chemical synthetics (imiquimod, cimetidine, levamisole, polyinosinic acid, pidotimod, and others); and biological cytokines (transfer factor, interferon, immune globulin, and interleukin) [68, 95, 97].

Bricknell and Dalmo [98] reported that the addition of immunostimulants in animal feed could enhance their innate defense and prevent infection during a period of high stress. Another group of researchers, Gertsch et al. [99], stated that applying plant-derived immunostimulants in animal feed boosts the immune system though they did not specify the mechanism. Similarly, Li et al. [100] administered polysaccharide chitosan to cattle and noted improved immune response and antioxidant activity. In 2010, Thacker [101] reported cytosine-phosphate-guanine (CpG), an oligo deoxynucleotides immune-stimulant, stimulating B-cell proliferation, cytokine production, and enhanced cytokines production and NK cell cytotoxic activity.

### 3.6 Cytokines

Cytokines are crucial for normal tissue functions, but their over- or under-expression is linked with pathological conditions [102]. They play a significant role in initiating, sustaining, and controlling the innate immune response and suggesting that they may have an excellent therapeutic effect for infectious disease treatment [103]. Toll-like receptors (TLRs), surface receptors that identify the structure of pathogens, also indirectly contribute to the secretion of cytokines by inducing a signaling cascade that leads to the secretion of cytokines controlling the adaptive immune response [104].

Cytokines, such as IL-6, TNF-α, and INF-γ, have also been proposed to treat bovine mastitis and endometritis. Hossain et al. [105] reported that cytokines alone or in combination with antibiotics significantly improve the rate of cure of bovine mastitis. Daley et al. [106] infused the mammary gland with recombinant bovine cytokines ((IL-1 and IL-2) and observed a rise in the proliferation of polymorphonuclear cells, with increased formation of oxygen radicals in the milk. The investigators also observed that the induced host natural defense system could prevent *S. aureus* infection in cattle. This suggests recombinant bovine cytokines are a promising candidate. Thus, further investigation is needed to identify the therapeutic potential of the cytokines for mastitis treatment and their possible use as an alternative to antibiotics.

### 3.7 Phage therapy

Phage therapy, which treats bacterial infections with bacteriophages, has been considered one strategy to manage mastitis [47]. Results of several studies showed that bacteriophages had antibacterial activity against a range of antibiotic-resistant bacteria with a considerable degree of specificity and potency [107]. Thus, the use of bacteriophages and their derivatives such as endolysins signifies a possible alternative for treating mastitis [108].

The bacteriophage works by inserting its genome into the bacterial cytoplasm, thereby the phage genome will incorporate itself into the host genome and reproduce along with the bacteria and produce endolysin, which break-down the bacterial cell wall and induce a cascade of bacterial lysis [108, 109]. Phages and endolysins are also known to destroy biofilms produced by major gram-positive and gram-negative mastitis pathogens, including *Staphylococcus* species*, E. coli, Klebsiella pneumonia,* and others [110].

Currently, interest in bacteriophages for the treatment of mastitis is rapidly growing [80]. Results from several *in vitro* experiments indicated that this method of treating mastitis is a viable option as phage therapy shows promising effectiveness against some mastitis pathogens, such as *S. aureus* [107, 108, 110–114]. However, a handful of clinical studies to evaluate the efficacy of bacteriophage for the treatment and prevention of mastitis showed limited efficacy of this approach, suggesting the

need for further study to improve its effectiveness [47, 112, 115]. Moreover, the practical use and broad application of phage therapy are limited by several factors. These include high specificity of phages, low effectiveness in eliminating the population of pathogenic bacteria, the need for a high dose of phage for effective therapy and its degradability in milk, and the emergence of phage resistance bacterial strains [108, 116]. Further clinical studies are needed to address these limitations and exploit the full potential of phage to prevent and treat mastitis.

### 3.8 Use of probiotics for the treatment of mastitis

The rise of AMR against antibiotics used in dairy farming demands the search for other alternative disease control measures. In this regard, probiotics have lately been considered a potential alternative for treating mastitis [49]. Probiotics are living microorganisms that give a health benefit to the recipient when given in sufficient amounts. This less precise definition includes several different well-identified microorganisms, safe for intended use, have proven health benefits when used in appropriate amounts and through the correct routes [117, 118].

Two mechanisms of action were suggested for mammary gland probiotics. The first mode of action is through the interactions between probiotics and the local microbiota (indirect mode) [48]. This model assumes that cows develop mastitis due to a lack of balance between the normal mammary gland microbiota and pathogenic bacteria causing mastitis. Therefore, modification of this imbalance with probiotics is suggested as an option to AMU [119]. The second proposed mode of action is a direct one, where probiotics interact directly with mastitis pathogen. Probiotic bacteria generate a range of antimicrobial substances such as short-chain fatty acids, lactic acid, nitric oxide, hydrogen peroxide, and bacteriocins, all of which may inhibit the growth and multiplication of mastitis-causing bacteria [120]. Rainard and Gilles [48] reviewed the use, mechanism of action, and *in vitro* and *in vivo* efficacy studies on probiotics used in mastitis treatment.

The selection and prophylactic or therapeutic use of mammary gland probiotic strains depend on the production of substances affecting the growth or survival of mastitis pathogens, the absence of known virulence factors, the absence of antibiotic resistance, and the ability to colonize mammary gland epithelium cells. The bacteria that meet these conditions are deemed promising for use as mammary probiotics [121]. Most studies investigated lactic acid bacteria as a potential probiotic for mastitis treatment and prevention. Few of these studies reported that probiotics are as effective as antibiotics for treating clinical mastitis [122]. In contrast, most other studies reported that the probiotics elicit a strong inflammatory response in the mammary gland or are neither effective nor safe [123, 124]. The current reports on the safety and efficacy of intramammary probiotics are generally conflicting, necessitating the need for further research to develop a conclusive recommendation on the use of probiotics for the management of mastitis.

### 3.9 Antimicrobial peptides

Antimicrobial peptides (AMPs), also known as cationic host defense peptides, are potent naturally occurring antibacterial agents with a broad spectrum of activities against both gram-negative and gram-positive bacteria. AMPs are found in all forms of life, from prokaryotes to eukaryotic cells. In contrast to most conventional antibiotics, AMPs often work in direct and indirect ways. They may directly kill the bacteria by disrupting cell membranes, thereby creating trans-membrane channels. They indirectly may also enhance host immunity as immunomodulators so that the host can clear the pathogen [49].

In vertebrates AMPs promote natural immunity and are a component of the first line of defense against pathogenic microorganisms. The crucial role of AMPs as innate immune modulators was shown in an experimental study in which the cnlp gene (encoding CRAMP) knockout mutant mice, a gene coding mouse analog of human LL-37 (encoded by camp) antimicrobial peptide, were very susceptible to infection [125]. In prokaryotes such as bacteria, the production and release of AMPs give a competitive advantage in a given environment by AMPs-mediated killing of other bacteria [126].

The mode of action of AMPs is recently reviewed [127] and seems different and related to the target bacterial pathogen. The positively charged AMPs interact with the negatively charged membranes of bacteria (lipopolysaccharides in gram-negative bacteria) and teichoic acids (in gram-positive bacteria). This strong electrostatic interaction between opposing charges (between AMPs and bacterial surface membranes) is the basis of the specificity of the action of AMPs on bacteria over other higher organisms. The "amphipathic" characteristics of AMPs help them to bind and penetrate the bacterial inner membrane causing leakage of bacterial cell contents and leading to cell death [128].

Currently, AMPs are considered as one of the promising classes of therapeutic agents as an alternative to conventional antibiotics. Several AMPs have been used as therapeutic agents for intravenous administration and topical application in human medicine owing to their short half-lives [129]. A recent study investigating the efficacy of specific AMPs against the AMR *S. aureus* in the mammary epithelial cells reported a very promising result. The study examined the intracellular activities of $H_2$ in the bovine mammary epithelial and mouse mammary glands infected with methicillin-resistant *S. aureus* (MRSA) and multidrug-resistant *S. aureus.* Results showed a 99% intracellular inhibition rate of the resistant *S. aureus* strains after treatment with the AMPs. The study finally concluded that $H_2$, the AMPs used in the study, "can be used as a safe and effective candidate for treating *S. aureus*-induced mastitis" [130]. This is an indication that AMPs-based treatment approaches may be used as one of the tools that may help in the fight against AMR pathogens. However, more studies are needed to generate information on the development of resistance to AMPs, challenges to their widespread use in dairy cattle.

### 3.10 Use of CRISPR-Cas system

The CRISPR-Cas system is a bacterial immune system that gives resistance to foreign genetic elements such as those that exist within plasmids and bacteriophages and provides a form of adaptive immunity [131]. In recent years, the use of the CRISPR-Cas system to treat AMR bacteria has received a considerable level of interest as the approach that can readily kill AMR bacteria in the same way as an antibiotic-sensitive bacterium [132]. Additionally, this system can be designed specifically so that it can only target pathogenic bacteria without disturbing commensal bacteria in the microbiota [50]. This bacterial immune system is commonly used for "genome editing" as it can selectively eliminate virulence and antimicrobial resistance genes from bacterial populations. The system uses small RNAs (sRNA) to detect and destroy specific sequences of DNA, including phages, transposons, and plasmids [133].

Nucleic acid-based antibacterial treatments can be used to control infections caused by resistant bacteria [134], including mastitis-causing pathogens. However, although *in vitro* studies on some resistant pathogens showed successful and promising results, *in vivo* study to treat mastitis pathogen has not yet been carried out [135]. Besides, despite its current potential, the sustainable application of CRISPR-Cas technology is complex. It needs an efficient delivery vector, developing an appropriate wide host

range vector, and using a multiplex method that includes CRISPR-Cas targeting different sequences to reduce the occurrence of resistance possibilities [136].

## 4. Conclusion

Mastitis is the most prevalent and economically important disease of dairy cattle responsible for the largest antibiotics used in the dairy industry. Most dairy farms in the United States use similar antibiotics used to treat various diseases in humans. Several studies have linked AMR to antibiotic use. Thus, the use of these classes of antibiotics in dairy cattle may speed up the development of AMR, which can also affect the successful treatment of infection in humans. Every effort must be made to avoid unnecessary use or reduce the use of antibiotics to prevent mastitis. Dairy farmers need to be educated on the importance of improving herd and udder health so that the incidence of clinical and subclinical mastitis will decrease, reducing the need to use antibiotics. The use of vaccines, probiotics, antimicrobial peptides, phage therapy, and CRISPR-Cas system are among the promising alternative options for mastitis management. To maintain dairy cattle health and productivity and preserve the effectiveness of antibiotics, these alternative approaches to antibiotic use must be thoroughly investigated and implemented for sustainable management of mastitis. *In vitro* studies showed promising results on the potential use of these approaches, but further *in vivo* studies are needed to make specific recommendations on their use. Research should focus on identifying good alternatives to antibiotics with important characteristics including but not limited to effectiveness against the target pathogens, safety toward the host, ease of elimination from the body, less harmful to normal flora, degradability in the environment, and cost. Thus, it is strongly recommended that researchers and funding organizations invest their resources and focus their effort on developing innovative and sustainable control tools that are easily adoptable by producers such as effective vaccines, probiotics, and others coupled with good herd health management practices.

## Author details

Benti D. Gelalcha[1], Getahun E. Agga[2] and Oudessa Kerro Dego[1*]

1 Department of Animal Science, The University of Tennessee, Knoxville, TN, USA

2 United States Department of Agriculture, Agricultural Research Service, Food Animal Environmental Systems Research Unit, Bowling Green, KY, USA

*Address all correspondence to: okerrode@utk.edu

## References

[1] Davies J, Davies D. Origins and evolution of antibiotic resistance. Microbiology and Molecular Biology Reviews. 2010;**74**:417-433

[2] CDDEP (Center for Disease Dynamics Economics and Policy). 2020. ResistanceMap: Antibiotic Resistance. 2020. Available from: https://resistancemap.cddep.org/AntibioticResistance.php [Accessed: November 1, 2021]

[3] ReAct. Antibiotic Use in Food Animals: India Overview. Vellore, India: Asia-Pacific Christian Medical College; 2018

[4] Pol M, Ruegg PL. Relationship between antimicrobial drug usage and antimicrobial susceptibility of gram-positive mastitis pathogens. Journal of Dairy Science. 2007;**90**:262-273

[5] CDC. Prevention. Atlanta, GA: CDC; 2017

[6] Pol M, Ruegg PL. Treatment practices and quantification of antimicrobial drug usage in conventional and organic dairy farms in Wisconsin. Journal of Dairy Science. 2007;**90**:249-261

[7] Barlow J. Mastitis therapy and antimicrobial susceptibility: A multispecies review with a focus on antibiotic treatment of mastitis in dairy cattle. Journal of Mammary Gland Biology and Neoplasia. 2011;**16**:383-407

[8] USDA APHIS. Milk Quality, Milking Procedures, and Mastitis on U.S. Dairies, Veterinary Services, National Animal Health Monitoring System. 2014. Avilable from: https://www.aphis.usda.gov/animal_health/nahms/dairy/downloads/dairy14/Dairy14_dr_Mastitis.pdf [Accessed: November 1, 2020]

[9] Ruegg PL. What is success? A narrative review of research evaluating outcomes of antibiotics used for treatment of clinical mastitis. Frontiers in Veterinary Science. 2021;**8**:639641

[10] Smith KL, Todhunter DA, Schoenberger PS. Environmental pathogens and intramammary infection during the dry period. Journal of Dairy Science. 1985;**68**:402-417

[11] Oliver SP. Frequency of isolation of environmental mastitis-causing pathogens and incidence of new intramammary infection during the nonlactating period. American Journal of Veterinary Research. 1988; **49**:1789-1793

[12] Oliver S, Mitchell B. Susceptibility of bovine mammary gland to infections during the dry period. Journal of Dairy Science. 1983;**66**:1162-1166

[13] Berry EA, Hillerton JE. The effect of selective dry cow treatment on new intramammary infections. Journal of Dairy Science. 2002;**85**:112-121

[14] Redding LE, Bender J, Baker L. Quantification of antibiotic use on dairy farms in Pennsylvania. Journal of Dairy Science. 2019;**102**:1494-1507

[15] FDA. 2014 Summary Report On Antimicrobials Sold or Distributed for Use in Food-Producing Animals. 2015. Available from: https://www.fda.gov/media/94906/download [Accessed: November 1, 2021]

[16] WHO. Critically Important Antimicrobials for Human Medicine. 5th Revision 2016. Ranking of Medically Important Antimicrobials for Risk Management of Antimicrobial Resistance Due to Non-Human Use. 2017. Available from: https://apps.who.int/iris/bitstream/handle/10665/255027/9789241512220-eng.pdf?sequence=1&isAllowed=y [Accessed: November 1, 2021]

[17] Doane M, Sarenbo S. Antibiotic usage in 2013 on a dairy CAFO in NY State, USA. Infection Ecology & Epidemiology. 2014;**4**:24259

[18] Oliver JP, Gooch C. Dairy Environmental Systems: Critically Important Antimicrobials Labeled for Dairy Use. 2017. Available from: https://ecommons.cornell.edu/bitstream/handle/1813/103791/FS%20-%20Antibiotics_DairyManure%20-%201.%20Critically%20imp.%20antimicrobials-VD.pdf?sequence=2&isAllowed=y [Accessed: November 1, 2021]

[19] FDA. 2019 Summary Report on Antimicrobials Sold or Distributed for Use in Food-Producing Animals. 2020. Available from: https://www.fda.gov/media/144427/download [Accessed: November 1, 2021]

[20] Aarestrup FM, Wegener HC, Collignon P. Resistance in bacteria of the food chain: Epidemiology and control strategies. Expert Review of Anti-Infective Therapy. 2008;**6**:733-750

[21] Anonymous. Antimicrobial resistance on dairy farms. Foodborne Pathogens and Disease. 2019;**16**:1-4

[22] O'Neill J. Antimicrobials in Agriculture and the Environment: Reducing Unnecessary Use and Waste. 2015. Available from: https://ec.europa.eu/health/sites/default/files/antimicrobial_resistance/docs/amr_studies_2015_am-in-agri-and-env.pdf [Accessed: November 1, 2021]

[23] Singer RS, Finch R, Wegener HC, Bywater R, Walters J, Lipsitch M. Antibiotic resistance-the interplay between antibiotic use in animals and human beings. The Lancet Infectious Diseases. 2003;**3**:47-51

[24] Lazarus B, Paterson DL, Mollinger JL, Rogers BA. Do human extraintestinal *Escherichia coli* infections resistant to expanded-spectrum cephalosporins originate from food-producing animals? A systematic review. Clinical Infectious Diseases. 2015;**60**:439-452

[25] Wall BA, Mateus A, Marshall L, Pfeiffer DU. Drivers, Dynamics and Epidemiology of Antimicrobial Resistance in Animal Production. Rome: FAO; 2016

[26] Juhász-Kaszanyitzky É, Jánosi S, Somogyi P, Dán Á, Van Bloois LV, Van Duijkeren E, et al. MRSA transmission between cows and humans. Emerging Infectious Diseases. 2007;**13**:630-632

[27] Schmidt T, Kock MM, Ehlers MM. Diversity and antimicrobial susceptibility profiling of staphylococci isolated from bovine mastitis cases and close human contacts. Journal of Dairy Science. 2015;**98**:6256-6269

[28] Smith TC. Livestock-associated *Staphylococcus aureus*: The United States experience. PLoS Pathogens. 2015;**11**:e1004564

[29] Del Collo LP, Karns JS, Biswas D, Lombard JE, Haley BJ, Kristensen RC, et al. Prevalence, antimicrobial resistance, and molecular characterization of *Campylobacter* spp. in bulk tank milk and milk filters from US dairies. Journal of Dairy Science. 2017;**100**:3470-3479

[30] Lejeune JT, Rajala-Schultz PJ. Food safety: Unpasteurized milk: A continued public health threat. Clinical Infectious Diseases. 2009;**48**:93-100

[31] Martin MJ, Thottathil SE, Newman TB. Antibiotics overuse in animal agriculture: A call to action for health care providers. American Journal of Public Health. 2015;**105**:2409-2410

[32] Mungai EA, Behravesh CB, Gould LH. Increased outbreaks associated with nonpasteurized milk,

United States, 2007-2012. Emerging Infectious Diseases. 2015;**21**:119-122

[33] Muloi D, Ward MJ, Pedersen AB, Fèvre EM, Woolhouse MEJ, Van Bunnik BAD. Are food animals responsible for transfer of antimicrobial-resistantescherichia colior their resistance determinants to human populations? A systematic review. Foodborne Pathogens and Disease. 2018;**15**:467-474

[34] Oliver SP, Murinda SE, Jayarao BM. Impact of antibiotic use in adult dairy cows on antimicrobial resistance of veterinary and human pathogens: A comprehensive review. Foodborne Pathogens and Disease. 2011;**8**:337-355

[35] Sato T, Okubo T, Usui M, Yokota S-I, Izumiyama S, Tamura Y. Association of veterinary third-generation cephalosporin use with the risk of emergence of extended-spectrum-cephalosporin resistance in *Escherichia coli* from dairy cattle in Japan. PLoS One. 2014;**9**:e96101

[36] Oliver SP, Boor KJ, Murphy SC, Murinda SE. Food safety hazards associated with consumption of raw milk. Foodborne Pathogens and Disease. 2009;**6**:793-806

[37] Berge AC, Baars T. Raw milk producers with high levels of hygiene and safety—CORRIGENDUM. Epidemiology and Infection. 2020;**148**:e77

[38] Abdi RD, Gillespie BE, Vaughn J, Merrill C, Headrick SI, Ensermu DB, et al. Antimicrobial resistance of *Staphylococcus aureus* isolates from dairy cows and genetic diversity of resistant isolates. Foodborne Pathogens and Disease. 2018;**15**:449-458

[39] Levy SB, Fitzgerald GB, Macone AB. Spread of antibiotic-resistant plasmids from chicken to chicken and from chicken to man. Nature. 1976;**260**:40-42

[40] Lago A, Godden SM, Bey R, Ruegg PL, Leslie K. The selective treatment of clinical mastitis based on on-farm culture results: II. Effects on lactation performance, including clinical mastitis recurrence, somatic cell count, milk production, and cow survival. Journal of Dairy Science. 2011;**94**:4457-4467

[41] Myllys V, Honkanen-Buzalski T, Huovinen P, Sandholm M, Nurmi E. Association af changes in the bacterial ecology of bovine mastitis with changes in the use of milking machines and antibacterial drugs. Acta Veterinaria Scandinavica. 1994;**35**:363-369

[42] Tikofsky LL, Barlow JW, Santisteban C, Schukken YH. A comparison of antimicrobial susceptibility patterns for *Staphylococcus aureus* in organic and conventional dairy herds. Microbial Drug Resistance. 2003;**9**(Suppl. 1):S39-S45

[43] Scott HM, Acuff G, Bergeron G, Bourassa MW, Gill J, Graham DW, et al. Critically important antibiotics: Criteria and approaches for measuring and reducing their use in food animal agriculture. Annals of the New York Academy of Sciences. 2019;**1441**:8-16

[44] Rabiee AR, Lean IJ. The effect of internal teat sealant products (Teatseal and Orbeseal) on intramammary infection, clinical mastitis, and somatic cell counts in lactating dairy cows: A meta-analysis. Journal of Dairy Science. 2013;**96**:6915-6931

[45] Dargatz DA, Garry FB, Traub-Dargatz JL. An introduction to biosecurity of cattle operations. The Veterinary Clinics of North America. Food Animal Practice. 2002;**18**(1-5):v

[46] Middleton JR. Vaccination against Staphylococcus Aureus Mastitis in Dairy Cattle. 2019. Available from: https://dairy-cattle.extension.org/

vaccination-against-staphylococcus-aureus-mastitis-in-dairy-cattle/ [Accessed: October 20, 2021]

[47] Gill JJ, Pacan JC, Carson ME, Leslie KE, Griffiths MW, Sabour PM. Efficacy and pharmacokinetics of bacteriophage therapy in treatment of subclinical *Staphylococcus aureus* mastitis in lactating dairy cattle. Antimicrobial Agents and Chemotherapy. 2006;**50**: 2912-2918

[48] Rainard P, Foucras G. A critical appraisal of probiotics for mastitis control. Frontiers in Veterinary Science. 2018;**5**:251

[49] Ageitos JM, Sánchez-Pérez A, Calo-Mata P, Villa TG. Antimicrobial peptides (AMPs): Ancient compounds that represent novel weapons in the fight against bacteria. Biochemical Pharmacology. 2017;**133**:117-138

[50] Aslam B, Rasool M, Idris A, Muzammil S, Alvi RF, Khurshid M, et al. CRISPR-Cas system: A potential alternative tool to cope antibiotic resistance. Antimicrobial Resistance & Infection Control. 2020;**9**:1-3

[51] Nickerson SC, Ryman VE. Role of Antibiotic Therapy in Mastitis Control for Lactating and Dry Cows. 2019. Available from: https://secure.caes.uga.edu/extension/publications/files/pdf/B%201516_1.PDF [Accessed: October 20, 2021]

[52] Armstrong J. Selective Dry Cow Therapy. 2020. Available from: https://extension.umn.edu/dairy-milking-cows/selective-dry-cow-therapy [Accessed: October 20, 2021]

[53] Berry EA, Hillerton JE. Effect of an intramammary teat seal and dry cow antibiotic in relation to dry period length on postpartum mastitis. Journal of Dairy Science. 2007;**90**:760-765

[54] Roberson JR. Establishing treatment protocols for clinical mastitis. The Veterinary Clinics of North America, Food Animal Practice. 2003;**19**:223-234

[55] Lago A, Godden SM, Bey R, Ruegg PL, Leslie K. The selective treatment of clinical mastitis based on on-farm culture results: I. Effects on antibiotic use, milk withholding time, and short-term clinical and bacteriological outcomes. Journal of Dairy Science. 2011;**94**:4441-4456

[56] Taponen S, Salmikivi L, Simojoki H, Koskinen MT, Pyorala S. Real-time polymerase chain reaction-based identification of bacteria in milk samples from bovine clinical mastitis with no growth in conventional culturing. Journal of Dairy Science. 2009;**92**:2610-2617

[57] van den Borne BH, Nielen M, Van Schaik G, Melchior MB, Lam TJ, Zadoks RN. Host adaptation of bovine Staphylococcus aureus seems associated with bacteriological cure after lactational antimicrobial treatment. Journal of Dairy Science. 2010; **93**:2550-2558

[58] Sandgren CH, Waller KP, Emanuelson U. Therapeutic effects of systemic or intramammary antimicrobial treatment of bovine subclinical mastitis during lactation. Veterinary Journal. 2008;**175**:108-117

[59] Oliver SP, Gillespie BE, Headrick SJ, Moorehead H, Lunn P, Dowlen HH, et al. Efficacy of extended ceftiofur intramammary therapy for treatment of subclinical mastitis in lactating dairy cows. Journal of Dairy Science. 2004;**87**:2393-2400

[60] Pankey JW, Barker RM, Twomey A, Duirs G. A note on effectiveness of dry cow therapy in New Zealand dairy herds. New Zealand Veterinary Journal. 1982;**30**:50-52

[61] Pankey JW, Barker RM, Twomey A, Duirs G. Comparative efficacy of

dry-cow treatment regimens against *Staphylococcus aureus*. New Zealand Veterinary Journal. 1982;**30**:13-15

[62] BAMN (Bovine Alliance on Management and Nutritio). An Introduction to Infectious Disease Control on Farms (Biosecurity). Arlington, VA: American Feed Industry Association (AFIA); 2001

[63] Haskell MJ, Rennie LJ, Bowell VA, Bell MJ, Lawrence AB. Housing system, milk production, and zero-grazing effects on lameness and leg injury in dairy cows. Journal of Dairy Science. 2006;**89**:4259-4266

[64] EFSA (European Food Safety Authority). Scientific opinion on welfare of dairy cows in relation to leg and locomotion problems based on a risk assessment with special reference to the impact of housing, feeding, management and genetic selection. The EFSA Journal. 2009;**1142**:1-57

[65] Strandberg E, Emanuelson U. Herd-level factors associated with longevity in Swedish dairy cattle. Acta Agriculturae Scandinavica, Section A—Animal Science. 2016;**66**:92-98

[66] Ismail ZB. Mastitis vaccines in dairy cows: Recent developments and recommendations of application. Veterinary World. 2017;**10**:1057

[67] Park KS, Sun X, Aikins ME, Moon JJ. Non-viral COVID-19 vaccine delivery systems. Advanced Drug Delivery Reviews. 2021;**169**:137-151

[68] Cheng G, Hao H, Xie S, Wang X, Dai M, Huang L, et al. Antibiotic alternatives: The substitution of antibiotics in animal husbandry? Frontiers in Microbiology. 2014;**5**:217

[69] Gast RK. Serotype-specific and serotype-independent strategies for preharvest control of food-borne salmonella in poultry. Avian Diseases. 2007;**51**:817-828

[70] Potter A, Gerdts V, Littel-van den Hurk S. Veterinary vaccines: Alternatives to antibiotics? Animal Health Research Reviews. 2008; **9**:187-199

[71] Bradley AJ, Breen J, Payne B, White V, Green MJ. An investigation of the efficacy of a polyvalent mastitis vaccine using different vaccination regimens under field conditions in the United Kingdom. Journal of Dairy Science. 2015;**98**:1706-1720

[72] Middleton JR, Luby CD, Adams DS. Efficacy of vaccination against staphylococcal mastitis: A review and new data. Veterinary Microbiology. 2009;**134**:192-198

[73] Middleton JR, Ma J, Rinehart CL, Taylor VN, Luby CD, Steevens BJ. Efficacy of different Lysigin formulations in the prevention of *Staphylococcus aureus* intramammary infection in dairy heifers. The Journal of Dairy Research. 2006;**73**:10-19

[74] Schukken Y, Bronzo V, Locatelli C, Pollera C, Rota N, Casula A, et al. Efficacy of vaccination on *Staphylococcus aureus* and coagulase-negative staphylococci intramammary infection dynamics in 2 dairy herds. Journal of Dairy Science. 2014;**97**:5250-5264

[75] Luby CD, Middleton JR. Efficacy of vaccination and antibiotic therapy against *Staphylococcus aureus* mastitis in dairy cattle. The Veterinary Record. 2005;**157**:89-90

[76] Luby CD, Middleton JR, Ma J, Rinehart CL, Bucklin S, Kohler C, et al. Characterization of the antibody isotype response in serum and milk of heifers vaccinated with a *Staphylococcus aureus* bacterin (Lysigin). The Journal of Dairy Research. 2007;**74**:239-246

[77] Smith GW, Lyman RL, Anderson KL. Efficacy of vaccination and antimicrobial treatment to eliminate chronic intramammary *Staphylococcus aureus* infections in dairy cattle. Journal of the American Veterinary Medical Association. 2006;**228**:422-425

[78] Piepers S, Prenafeta A, Verbeke J, De Visscher A, March R, De Vliegher S. Immune response after an experimental intramammary challenge with killed *Staphylococcus aureus* in cows and heifers vaccinated and not vaccinated with Startvac, a polyvalent mastitis vaccine. Journal of Dairy Science. 2017; **100**:769-782

[79] Freick M, Frank Y, Steinert K, Hamedy A, Passarge O, Sobiraj A. Mastitis vaccination using a commercial polyvalent vaccine or a herd-specific *Staphylococcus aureus* vaccine. Tierärztliche Praxis G: Großtiere/ Nutztiere. 2016;**44**:219-229

[80] Landin H, Mork MJ, Larsson M, Waller KP. Vaccination against *Staphylococcus aureus* mastitis in two Swedish dairy herds. Acta Veterinaria Scandinavica. 2015;**57**:81

[81] Merrill C, Ensermu DB, Abdi RD, Gillespie BE, Vaughn J, Headrick SI, et al. Immunological responses and evaluation of the protection in dairy cows vaccinated with staphylococcal surface proteins. Veterinary Immunology and Immunopathology. 2019;**214**:109890

[82] Kerro Dego O, Almeida R, Ivey S, Agga GE. Evaluation of *Streptococcus uberis* surface proteins as vaccine antigens to control S. uberis mastitis in dairy cows. MDPI Vaccines. 2021;**9**:868

[83] Collado R, Montbrau C, Sitja M, Prenafeta A. Study of the efficacy of a *Streptococcus uberis* mastitis vaccine against an experimental intramammary infection with a heterologous strain in dairy cows. Journal of Dairy Science. 2018;**101**:10290-10302

[84] Wilson DJ, Grohn YT, Bennett GJ, González RN, Schukken YH, Spatz J. Comparison of J5 vaccinates and controls for incidence, etiologic agent, clinical severity, and survival in the herd following naturally occurring cases of clinical mastitis. Journal of Dairy Science. 2007;**90**:4282-4288

[85] Wilson DJ, Mallard BA, Burton JL, Schukken YH, Grohn YT. Association of *Escherichia coli* J5-specific serum antibody responses with clinical mastitis outcome for J5 vaccinate and control dairy cattle. Clinical and Vaccine Immunology. 2009;**16**:209-217

[86] Hogan JS, Smith KL, Todhunter DA, Schoenberger PS. Field trial to determine efficacy of an *Escherichia coli* J5 mastitis vaccine. Journal of Dairy Science. 1992;**75**:78-84

[87] Almeida RA, Matthews KR, Cifrian E, Guidry AJ, Oliver SP. *Staphylococcus aureus* invasion of bovine mammary epithelial cells. Journal of Dairy Science. 1996;**79**:1021-1026

[88] Misra N, Wines TF, Knopp CL, Hermann R, Bond L, Mitchell B, et al. Immunogenicity of a *Staphylococcus aureus*-cholera toxin A2/B vaccine for bovine mastitis. Vaccine. 2018; **36**:3513-3521

[89] Nickerson SC, Owens WE, Tomita GM, Widel P. Vaccinating dairy heifers with a *Staphylococcus aureus* bacterin reduces mastitis at calving. Large Animal Practice. 1999;**20**:16-28

[90] Williams JM, Mayerhofer HJ, Brown RW. Clinical evaluation of a *Staphylococcus aureus* bacterin (polyvalent somatic antigen). Veterinary Medicine, Small Animal Clinician. 1966;**61**:789-793

[91] Williams JM, Shipley GR, Smith GL, Gerber DL. A clinical evaluation of

*Staphylococcus aureus* bacterin in the control of staphylococcal mastitis in cows. Veterinary Medicine, Small Animal Clinician. 1975;**70**:587-594

[92] Yancey RJ. Recent advances in bovine vaccine technology. Journal of Dairy Science. 1993;**76**:2418-2436

[93] Tashakkori N, Khoramian B, Farhoodi Moghadam M, Heidarpour M, Mashayekhi K, Farzaneh N. Evaluating the effectiveness of two bovine mastitis vaccines and their influences on oxidant and antioxidant capacities of milk. Tropical Animal Health and Production. 2020;**52**:1493-1501

[94] Sakai M. Current research status of fish immunostimulants. Aquaculture. 1999;**172**:63-92

[95] Song SK, Beck BR, Kim D, Park J, Kim J, Kim HD, et al. Prebiotics as immunostimulants in aquaculture: A review. Fish & Shellfish Immunology. 2014;**40**:40-48

[96] Sharma C, Rokana N, Chandra M, Singh BP, Gulhane RD, Gill JPS, et al. Antimicrobial resistance: Its surveillance, impact, and alternative management strategies in dairy animals. Frontiers in Veterinary Science. 2017;**4**:237

[97] Masihi KN. Immunomodulators in infectious diseases: Panoply of possibilites. International Journal of Immunopharmacology. 2000; **22**:1083-1091

[98] Bricknell I, Dalmo RA. The use of immunostimulants in fish larval aquaculture. Fish & Shellfish Immunology. 2005;**19**:457-472

[99] Gertsch J, Viveros-Paredes JM, Taylor P. Plant immunostimulants—Scientific paradigm or myth? Journal of Ethnopharmacology. 2011;**136**:385-391

[100] Li T, Na R, Yu P, Shi B, Yan S, Zhao Y, et al. Effects of dietary supplementation of chitosan on immune and antioxidative function in beef cattle. Czech Journal of Animal Science. 2016;**60**:38-44

[101] Thacker E. Immunomodulators, immunostimulants, and immunotherapies in small animal veterinary medicine. The Veterinary Clinics of North America Small Animal Practice. 2010;**40**(3):473-483

[102] Zhang J-M, An J. Cytokines, inflammation, and pain. International Anesthesiology Clinics. 2007;**45**:27-37

[103] Dhama K, Chakraborty S, Wani MY, Tiwari R, Barathidasan R. Cytokine therapy for combating animal and human diseases. A review. Research Opinions in Animal & Veterinary Sciences. 2013;**3**:195-208

[104] Takeda K, Akira S. Toll-like receptors in innate immunity. International Immunology. 2005; **17**(1):1-14

[105] Hossen MJ, Baek KS, Kim E, Yang WS, Jeong D, Kim JH, et al. In vivo and in vitro anti-inflammatory activities of Persicaria chinensis methanolic extract targeting Src/Syk/NF-kappaB. Journal of Ethnopharmacology. 2015;**159**:9-16

[106] Daley MJ, Williams TJ, Coyle P, Furda GJ, Dougherty R, Hayes PW. Prevention and treatment of *Staphylococcus aureus* infections with recombinant cytokines. Cytokine. 1993;**5**(3):276-284

[107] Porter J, Anderson J, Carter L, Donjacour E, Paros M. In vitro evaluation of a novel bacteriophage cocktail as a preventative for bovine coliform mastitis. Journal of Dairy Science. 2016;**99**:2053-2062

[108] Zduńczyk S, Janowski T. Bacteriophages and associated endolysins in therapy and prevention of

mastitis and metritis in cows: Current knowledge. Animal Reproduction Science. 2020;**218**:106504

[109] Sabino J, Hirten RP, Colombel J-F. Review article: Bacteriophages in gastroenterology-from biology to clinical applications. Alimentary Pharmacology & Therapeutics. 2020;**51**:53-63

[110] O'Flaherty S, Coffey A, Meaney WJ, Fitzgerald GF, Ross RP. Inhibition of bacteriophage K proliferation on *Staphylococcus aureus* in raw bovine milk. Letters in Applied Microbiology. 2005;**41**:274-279

[111] O'Flaherty S, Coffey A, Meaney W, Fitzgerald GF, Ross RP. The recombinant phage lysin lysk has a broad spectrum of lytic activity against clinically relevant staphylococci, including methicillin-resistant *Staphylococcus aureus*. Journal of Bacteriology. 2005;**187**:7161-7164

[112] Synnott AJ, Kuang Y, Kurimoto M, Yamamichi K, Iwano H, Tanji Y. Isolation from sewage influent and characterization of novel *Staphylococcus aureus* bacteriophages with wide host ranges and potent lytic capabilities. Applied and Environmental Microbiology. 2009;**75**:4483-4490

[113] Han JE, Kim JH, Hwang SY, Choresca CH Jr, Shin SP, Jun JW, et al. Isolation and characterization of a myoviridae bacteriophage against *Staphylococcus aureus* isolated from dairy cows with mastitis. Research in Veterinary Science. 2013;**95**:758-763

[114] Breyne K, Honaker RW, Hobbs Z, Richter M, Żaczek M, Spangler T, et al. Efficacy and safety of a bovine-associated staphylococcus aureus phage cocktail in a murine model of mastitis. Frontiers in Microbiology. 2017;**8**:2348

[115] García P, Madera C, Martínez B, Rodríguez A, Evaristo SJ. Prevalence of bacteriophages infecting *Staphylococcus aureus* in dairy samples and their potential as biocontrol agents. Journal of Dairy Science. 2009;**92**:3019-3026

[116] Oechslin F. Resistance development to bacteriophages occurring during bacteriophage therapy. Viruses. 2018;**10**:351

[117] Hill C, Guarner F, Reid G, Gibson GR, Merenstein DJ, Pot B, et al. The international scientific association for probiotics and prebiotics consensus statement on the scope and appropriate use of the term probiotic. Nature Reviews Gastroenterology & Hepatology. 2014;**11**:506-514

[118] Sanders ME, Benson A, Lebeer S, Merenstein DJ, Klaenhammer TR. Shared mechanisms among probiotic taxa: Implications for general probiotic claims. Current Opinion in Biotechnology. 2018;**49**:207-216

[119] Oikonomou G, Machado VS, Santisteban C, Schukken YH, Bicalho RC. Microbial diversity of bovine mastitic milk as described by pyrosequencing of metagenomic 16s rDNA. PLoS One. 2012;**7**:e47671

[120] Lebeer S, Bron PA, Marco ML, Van Pijkeren J-P, O'Connell Motherway M, Hill C, et al. Identification of probiotic effector molecules: Present state and future perspectives. Current Opinion in Biotechnology. 2018;**49**:217-223

[121] Espeche MC, Pellegrino M, Frola I, Larriestra A, Bogni C, Nader-Macías ME. Lactic acid bacteria from raw milk as potentially beneficial strains to prevent bovine mastitis. Anaerobe. 2012;**18**:103-109

[122] Klostermann K, Crispie F, Flynn J, Ross RP, Hill C, Meaney W. Intramammary infusion of a live culture of *Lactococcus lactis* for treatment of bovine mastitis: Comparison with antibiotic treatment in field trials.

Journal of Dairy Research. 2008; **75**:365-373

[123] Greene WA, Gano AM, Smith KL, Hogan JS, Todhunter DA. Comparison of probiotic and antibiotic intramammary therapy of cattle with elevated somatic cell counts. Journal of Dairy Science. 1991;**74**:2976-2981

[124] Crispie F, Alonso-Gómez M, O'Loughlin C, Klostermann K, Flynn J, Arkins S, et al. Intramammary infusion of a live culture for treatment of bovine mastitis: Effect of live lactococci on the mammary immune response. Journal of Dairy Research. 2008;**75**:374-384

[125] Nizet V, Ohtake T, Lauth X, Trowbridge J, Rudisill J, Dorschner RA, et al. Innate antimicrobial peptide protects the skin from invasive bacterial infection. Nature. 2001;**414**:454-457

[126] Hassan M, Kjos M, Nes IF, Diep DB, Lotfipour F. Natural antimicrobial peptides from bacteria: Characteristics and potential applications to fight against antibiotic resistance. Journal of Applied Microbiology. 2012;**113**:723-736

[127] Mookherjee N, Anderson MA, Haagsman HP, Davidson DJ. Antimicrobial host defence peptides: Functions and clinical potential. Nature Reviews Drug Discovery. 2020; **19**:311-332

[128] Sochacki KA, Barns KJ, Bucki R, Weisshaar JC. Real-time attack on single *Escherichia coli* cells by the human antimicrobial peptide LL-37. Proceedings of the National Academy of Sciences. 2011;**108**:E77-E81

[129] Gomes B, Augusto MT, Felício MR, Hollmann A, Franco OL, Gonçalves S, et al. Designing improved active peptides for therapeutic approaches against infectious diseases. Biotechnology Advances. 2018;**36**:415-429

[130] Wang X, Teng D, Wang X, Hao Y, Chen H, Mao R, et al. Internalization, distribution, and activity of peptide H2 against the intracellular multidrug-resistant bovine mastitis-causing bacterium *Staphylococcus aureus*. Scientific Reports. 2019;**9**(1):7968. DOI: 10.1038/s41598-019-44459-x

[131] Barrangou R, Fremaux C, Deveau HLN, Richards M, Boyaval P, Moineau S, et al. CRISPR provides acquired resistance against viruses in prokaryotes. Science. 2007;**315**: 1709-1712

[132] Gholizadeh P, Köse Ş, Dao S, Ganbarov K, Tanomand A, Dal T, et al. How CRISPR-Cas system could be used to combat antimicrobial resistance. Infection and Drug Resistance. 2020;**13**:1111-1121

[133] Brouns SJJ, Jore MM, Lundgren M, Westra ER, Slijkhuis RJH, Snijders APL, et al. Small CRISPR RNAs guide antiviral defense in prokaryotes. Science. 2008;**321**:960-964

[134] De La Fuente-Nunez C, Torres MD, Mojica FJ, Lu TK. Next-generation precision antimicrobials: Towards personalized treatment of infectious diseases. Current Opinion in Microbiology. 2017;**37**:95-102

[135] Bikard D, Euler CW, Jiang W, Nussenzweig PM, Goldberg GW, Duportet X, et al. Exploiting CRISPR-Cas nucleases to produce sequence-specific antimicrobials. Nature Biotechnology. 2014;**32**:1146-1150

[136] Pursey E, Sünderhauf D, Gaze WH, Westra ER, Van Houte S. CRISPR-Cas antimicrobials: Challenges and future prospects. PLoS Pathogens. 2018;**14**:e1006990